HER2 TESTS: How Do We Choose?

For women with breast cancer, a faulty test defining their HER2 status can alter their treatment course while adding to costs. Getting it right has huge potential for not only saving payers their money, but also saving women their lives.

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When my sister was diagnosed with breast cancer in 2006, my job was to get answers to such questions as "How reliable is an immunohistochemical HER2 test?"

The breast oncology nurse at the Indiana University Medical Group who took my call didn't even ask about my sister's immunohistochemical (IHC) HER2 test results. Sounding very much like she had answered this question before, she told me, "The test needs to be confirmed by a FISH test," and went on to explain that fluorescence in situ hybridization (FISH) was the definitive HER2 test.

My sister eventually decided on a double mastectomy and reconstructive surgery, but that conversation with the oncology nurse came back to me when I read a 2007 *New York Times* article titled "Cancer Drug May Elude Many Women Who Need It" (Pollack 2007).

The article reported on two studies that called into question the reliability of *both* IHC and FISH tests for overexpression of the HER2

"Many, many variables can influence the result" of an immunohistochemistry HER2 assay, says Soonmyung Paik, MD, the principal investigator of the National Surgical Adjuvant Breast and Bowel Project Trial B-31.

gene in breast cancer. Both studies were presented at the June 2007 meeting of the American Society of Clinical Oncology, in Chicago.

The prevailing mood among the assembled docs and researchers was summed up by Marc L. Citron, MD, a breast oncologist in Lake Success, N.Y., and professor at Albert Einstein College of Medicine, in the Bronx. Referring to HER2 overexpression in breast cancer, the article quoted Citron as saying, "Here we are 10 years into it, and we don't know how to test for it."

AN UNPRECEDENTED SURPRISE

Both IHC and FISH assays test breast tumor tissue for overexpression or amplification of human epidermal growth factor receptor 2, commonly referred to as HER2. Most women whose tumors test positive for HER2 gene amplification — approximately 30 percent of breast cancer diagnoses — respond well to chemotherapy plus trastuzumab (Herceptin), a monoclonal antibody that binds to the HER2 protein receptors on cancer cells and cancels the instructions that tell the cells to keep growing and dividing.

Developed by Genentech, trastuzumab was approved by the U.S. Food and Drug Administration in 1998, and is considered the first of the new generation of "personalized medicines" that are paired with a pharmacogenetic test (in this case, a HER2 assay).

One of the studies at the ASCO meeting was presented by Soonmyung Paik, MD, a pathologist and researcher at the National Surgical Adjuvant Breast and Bowel Project (NSABP) in Pittsburgh and principal investigator of NSABP Trial B-31. Edith A. Perez, MD, director of the Mayo Clinic Breast Clinic in Jacksonville, Fla., and principal investigator of Breast Intergroup Trial N9831, presented the other study.

The two studies have much in common. Both found that a significant percentage of women whose breast tumors tested HER2-positive by laboratories in the cities where the women were first diagnosed turned out later to be HER2-negative when tested by the "central labs" that retested tissue samples of all enrollees after the trials ended.

In Paik's trial, 18 percent of the locally tested HER2-positive specimens were found to be HER2-negative by central-lab testing in an initial pretrial screening. Entry criteria were tightened, and when each enrollee's tumor tissue specimen was central-lab tested, using both IHC and FISH assays at the end of the trial, 9.7 percent of the enrollees tested HER2-negative.

In the estimation of Jeffrey S. Ross, MD, 3 to 4 percent of all immunohistochemistry assays in the U.S. generate a false negative and are not followed by a FISH test.

Discrepancies between test results from local labs and central labs are common. What made news this time was that, in both trials, even HER2-negative women in the adjuvant setting (those whose tumors were surgically excised before enrolling in the trial) seem to have benefited from being treated with trastuzumab. This was unprecedented. Trastuzumab is considered effective only in HER2-positive women with metastatic breast cancer, not in the adjuvant setting.

"There are two issues here," says Paik, summing up the significance of the two studies. "One is how reproducible currently available HER2 tests are. Number two is whether HER2 is a valid marker to decide whether you're going to use Herceptin or not."

Paik further stresses that the reliability of both FISH and IHC results are called into question if unqualified labs perform the tests.

CULMINATION OF CONCERNS

Improving the accuracy of HER2 testing is exactly what ASCO and the College of American Pathologists had in mind when they released their "Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer." Authored by a joint ASCO/CAP expert panel that included Paik and Perez, the 28-page document was published simultaneously in the January 2007 issues of the *Journal of Clinical Oncology* and *Archives of Pathology & Laboratory Medicine* (Wolff 2007a, Wolfe 2007b).

The guideline recommendations are an attempt to address concerns about the accuracy of HER2 testing, concerns that predate Paik's and Perez's studies by several years. Those concerns are substantiated by the guideline recommendations, which concede that "Approximately 20 percent of current HER2 testing may be inaccurate."

One reason why the accuracy of HER2 testing deteriorated in the first place may be economics. A lab can buy commercially available, FDA-approved HER2 test kits, or it can develop its own in-house HER2 test, also known as a *home brew.* This is a perfectly legitimate strategy, provided the lab makes sure

A false HER2-positive comes with a price: 52 weeks of chemotherapy and trastuzumab exceeds $50,000 plus the expense of relieving the side effects.

that the results of its home brew test are "concordant" with an FDA-approved test kit.

"The strategy for doing that is, without doubt, to save money, because it's far cheaper to make your own home brew than to buy the packaged FDA-approved kits," says Jeffrey S. Ross, MD, Cyrus Strong Merrill professor and chair of the Department of Pathology and Laboratory Medicine at Albany Medical Center in Albany, N.Y. He is one of the developers of the FISH test and has conducted central-lab work for several neoadjuvant trastuzumab trials.

Ross, who specializes in breast and genitourinary pathology, enumerates three of the many ways even an FDA-approved HER2 test kit in a central lab can generate an incorrect result:

- A second assay on a new slice of formalin-fixed, paraffin-embedded tissue that comes from a different part of the tumor may generate a different result. Although infrequent, tumors can be HER2-positive in some places and HER2-negative in others.
- Sometimes, the whole chromosome on which the HER2 gene is located (along with thousands of other genes) is amplified in the tumor tissue. It's called chromosome 17 polysomy, and it can be misinterpreted as HER2 gene amplification.
- A technical error in an IHC assay (for instance, the dispenser that releases a drop of staining reagent on the slide fails to open) can be detected only by including a tissue specimen known to score 3+ on the same slide as the tissue being tested.[1] If such a control specimen is not used, an absence of staining can be misinterpreted as a negative test result. FISH assays have a built-in control to prevent false negatives.

Experienced pathologists know how to avoid these pitfalls, but most HER2 assays are not done by experienced pathologists. The ASCO/CAP Guideline Recommendations are designed to eliminate these and other sources of variation in HER2 testing by codifying validation of "home brew" tests and standardizing operating procedures, and by complying with new testing criteria to be monitored through the use of stringent laboratory accreditation standards, proficiency testing, and competency assessment. The guideline recommendations are being implemented and enforced by CAP.

One thing the guideline recommendations don't do is give the nod to either IHC or FISH. In fact, ASCO/CAP go out of their way to avoid any preference. In the results section of the abstract, the authors write: "When carefully validated testing is performed, available data do not clearly demonstrate the superiority of either immunohistochemistry (IHC) or in situ hybridization (ISH) as a predictor of benefit from anti-HER2 therapy."

Such even-handedness, though, is not supported by the evidence, according to one dissenting author of the guideline recommendations. A brief look at the difference between IHC and FISH may help to clarify this difference of opinion.

IHC VERSUS FISH

In an IHC assay, a slice of tumor tissue is stained, along with a 3+ control specimen in a corner of the slide, and then the tumor sample in question is examined with a bright field microscope. The amount of observed staining correlates with the quantity of HER2 protein.

Subjective decisions in scoring a tissue specimen are opportunities for variability in an IHC HER2 assay. Even flawless technique and interpretation can still generate a FISH result in what pathologists call the "indeterminate" range of 1.8 to 2.2, which means another slice of the tumor tissue has to be tested using a FISH assay.

"With immunohistochemistry, the results can vary based on how long the tissue is fixed and what antibody you use for the staining. Many, many variables can influence the result," says Paik, who nevertheless agrees with the majority of the ASCO/CAP expert panel that FISH is not superior to IHC. "You also have to judge the scoring based on how strong the staining is, and that can be quite subjective. You could have two pathologists looking

[1] A tumor tissue is scored 3+ when it has a "full basket weave" appearance (a series of dark brown touching circles around the cells) in at least 30 percent of the sample. If the basket weave pattern has "holes" in it — for example, if the circles don't extend 360 degrees around the cells or if the pattern comprises less than 30 percent of the total specimen — it's scored a 2+. A weak staining pattern drops the score to 1+, and no visible staining is a 0.

at the same slide and one might call it 2+ positive staining and one might call it 3+ positive staining."

Interpreting a FISH HER2 test, on the other hand, is a much more objective process, FISH proponents argue. With the FISH assay, the pathologist counts actual copies of HER2 genes, which appear as a red "signal" in a blue-stained cancer cell nucleus seen through the microscope.

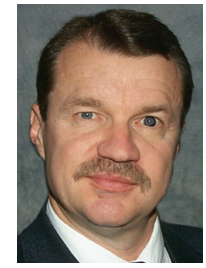
"On a good day, I can count to 20 and I can tell whether there are two copies of a red signal in a blue nucleus or whether there are 20 red signals in a blue nucleus," says Michael F. Press, MD, PhD, professor of pathology at the Keck School of Medicine, and coordinator of the Women's Cancers Program at the Norris Comprehensive Cancer Center, University of Southern California.

Press and his group at USC, in collaboration with a group led by Dennis J. Slamon, MD, PhD, who led the research for trastuzumab and is now director of the Revlon/UCLA Women's Cancer Research Program at Jonsson Comprehensive Cancer Center, in Los Angeles, and chief of the Division of Hematology/Oncology at UCLA's Department of Medicine, have amassed more than 20 years of published research on the topic of HER2 testing and the accuracy of IHC and FISH assays. Overall, these studies unambiguously point to FISH as consistently more accurate.

In daily practice, however, between 80 and 90 percent of primary HER2 testing in the United States is done with IHC, while only 10 to 20 percent is done with FISH. Approximately 10 percent of IHC test results fall into the so-called "indeterminate" range, and those specimens are re-tested using FISH.

If you're wondering why not just do the FISH test in the first place, you're not alone.

"That would be my position exactly," says Press, who then goes on to suggest several reasons why IHC outnumbers FISH in primary HER2 testing: Because pathologists are familiar with the assay (it's been in use since the 1970s); because many pathologists believe that an IHC assay is just as accurate as a FISH assay; and because it's fast and relatively inexpensive. Prices vary, but an IHC assay may cost $100 to $150, and a FISH assay may be double or triple that price.



"I do not think that immunohistochemistry done even in the best laboratories and with the best pathologists is good enough," says ASCO/CAP guideline co-author Michael F. Press, MD, PhD, "because the method is flawed."

Price certainly is a consideration, but incorrect HER2 test results entail far greater economic and human costs. A 52-week course of chemotherapy plus trastuzumab based on a false positive assay exceeds $50,000 and comes with a grab bag of nasty side effects, including potential cardiotoxicity. Conversely, a false negative assay deprives a woman with HER2-positive breast cancer of therapy that can offer a total pathologically complete response to treatment (complete disappearance of tumors from both the breast and lymph nodes) in nearly half of patients.

Ross estimates that approximately 3 to 4 percent of IHC assays in the United States generate a false negative and are not followed by a FISH test. Of these 3,000 to 4,000 women, 1,500 to 2,000 whom otherwise might have benefited from trastuzumab therapy will relapse with breast cancer.

"Labs like mine believe the FISH test is more reliable and accurate, so we don't bother with the immunohistochemistry and just do the definitive tests for all the patients," say Ross.

The accuracy of an IHC assay also is more dependent than FISH on preanalytic variables, such as how long it takes before the tissue specimen is fixed, how long it remains in the fixative solution, and how it's subsequently processed. In the United States, the majority of pathology specimens are fixed in formalin and embedded in paraffin.

"In 1989, we showed with molecularly characterized samples that immunohistochemistry has the potential for erroneously classifying tumors based on formalin-fixed, paraffin-embedded samples, whereas if one used a frozen tissue sample from the same patient, you got a relatively accurate result," says Press. "Formalin fixing and paraffin embedding introduce a lot of artifacts that confound the assay results. It's very hard to know whether you're getting a good result or a flawed result."

To be sure, FISH is not without its disadvantages. Cost again becomes a consideration because FISH requires a fluorescence microscope, a dark room in which to use it, and a board-certified pathologist who can tell the difference between the blue nuclei of cancer cells in the specimen and the blue nuclei of benign reactive cells.

FLAWED METHOD

As painstaking and as well-intentioned as the guideline recom-

As many as 2,000 women a year whose IHC test resulted in a false HER2-negative and who might have benefitted from trastuzumab therapy will relapse with breast cancer, according to one expert.

mendations are, there is reason to believe that they have missed an opportunity to raise the quality bar for HER2 assays even higher.

Press is a coauthor of the ASCO/CAP Guideline Recommendations, and he agrees with "95 percent" of the finished document.

"However, I don't agree with the ASCO/CAP Guidelines that suggest that immunohistochemistry is OK," says Press. "I do not think that immunohistochemistry done even in the best laboratories and with the best pathologists is good enough, because the method is flawed."

This is hardly news, at least not after 20 years of research published in peer-reviewed journals. Even more curious is that consumer Web sites such as «www.breastcancer.org» repeat Press's and Ross's assertions almost verbatim. Here's an excerpt from this site's text on Immunohistochemistry under "Will Herceptin Work for You?":

> "The IHC test results are most reliable for fresh or frozen tissue samples. IHC tends to be an unreliable way to test tissue that's preserved in wax or other chemicals. FISH testing is the preferred way to assess preserved tissue samples."[2]

Genentech's «www.Herceptin.com» site includes the following under FAQs:

> **Q. Which variables can affect the performance of IHC and FISH?**
> **A:** IHC detects HER2 overexpression at the protein level, and may be affected by conditions of the testing procedures. These include: time to fixation, duration of fixation, processing, denaturation, heating, antigen retrieval, the staining procedure used, and the interpretation of staining. Although there are antigen retrieval techniques in use, these may result in false-positive IHC results. FISH measures HER2 DNA. Some fixatives, chemicals or heat, may interfere with the FISH assay. However, an internal control is used to distinguish between a FISH-negative and a non-informative result.[3]

The ASCO/CAP Guideline Recommendations mention a newly available bright field [as opposed to fluorescent] ISH assay and a new chromogenic assay in the FDA pipeline. Until these new assays prove themselves in practice, surgeons and radiologists will continue to send tissue samples for IHC and FISH HER2 testing, and breast oncologists will continue to weigh the results along with other prognostic factors in planning treatment for their patients.

Citron, the breast oncologist from Einstein College of Medicine, is sticking by his initial reaction at the 2007 ASCO meeting that we still don't know how to test for HER2. He's also not convinced that FISH is any kind of gold standard, pointing out that "Table 2 of the package insert for Herceptin shows IHC predicts better than FISH for response to the drug."

Citron is concerned, however, that the ASCO/CAP Guideline Recommendations appear to have eliminated the possibility of false positive HER2 test results, regardless of which assay is used.

"The problem is that clinicians may get discrepant values, for example, between a patient who tests positive on core [biopsy], and then is negative on the specimen for IHC and for FISH," Citron explains. "According to the ASCO/CAP Guidelines, any patient who tests positive either by IHC or by FISH is a candidate for trastuzumab. It's possibly a false positive, but there's no way to prove that right now, and those patients end up being treated as HER2-positive patients."

[2] «http://www.breastcancer.org/treatment/targeted_therapies/herceptin/will_it_work.jsp»

[3] «http://www.herceptin.com/herceptin/professional/testing/faqs.jsp»

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